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The exponential model for a regulatory enzyme

An interpretation of the linear free-energy relationship

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A physical mechanism is suggested to explain the linear free-energy relationship employed in the exponential model for a regulatory enzyme [Ainsworth (1977) J. Theor. Biol. **68**, 391–413]. The interpretation depends on the assumption that the structure of the enzyme changes in proportion to its saturation by substrate but at a rate that is low compared with the rates of the association–dissociation reactions of the enzyme–substrate system.

INTRODUCTION

The exponential model for a regulatory enzyme with one substrate is represented by the equations:

$$p_i = \frac{v_i}{V} = \frac{A_i \alpha_i}{1 + A_i \alpha_i} \quad (1)$$

and

$$-\Delta G_i^0 = kp_i = \ln\left(\frac{\alpha_i}{\alpha_0}\right) \quad (2)$$

where p_i is the fractional saturation of the enzyme by substrate A, equal to v_i/V, the ratio of the initial velocity of the catalysed reaction at concentration A_i to the maximum velocity observed when A is raised to a saturating concentration. The affinity constant, α_i, is related to the fractional saturation, p_i, by a constant k, equal to $\ln(\alpha_1/\alpha_0)$ where α_1 and α_0 respectively are values of the affinity constant corresponding to $p = 1$ and $p = 0$. The change in affinity of the enzyme for A, as p_i varies, is supposed to arise from a change in the structure of the protein represented by a change in free energy $-\Delta G_i^0$. Both ΔG_i^0 and k are free-energy differences given in RT units.

Eqns. (1) and (2) (or their equivalents) have been suggested independently as models for regulatory behaviour on at least four occasions (Thompson & Klotz, 1971; Sturgill & Biltonen, 1976; Ainsworth, 1977; Whitehead, 1978), but the systematic investigation of their use has been limited to the studies by Ainsworth and co-workers (Ainsworth, 1977, 1979; Kinderlerer & Ainsworth, 1978; Gregory & Ainsworth, 1981*a*,*b*). When the equations were extended for use with two or more ligands (substrates and effector) a wider range of data became available for examination (Ainsworth & Gregory, 1978; Ainsworth *et al.*, 1981, 1983; Gregory *et al.*, 1983; Morris *et al.*, 1984, 1986; Ainsworth & Kinderlerer, 1984; Kinderlerer *et al.*, 1986; Rhodes *et al.*, 1986; Ainsworth, 1986). As a result of these studies it can be concluded that the exponential model is capable of describing a wide range of regulatory behaviour by a relatively limited number of constants. It should be noted that the conclusion is not extended to suggest that the success of the description proves the validity of the model.

The physical basis for the model has received relatively little attention. Sturgill & Biltonen (1976) and Whitehead (1978) suggested eqns. (1) and (2) on purely empirical grounds. Thompson & Klotz (1971), however, developed the equations by an Ising model approach: in particular, they assumed that the interaction between binding sites is very long-ranged and the number of binding sites sufficiently large, so that each site experiences a 'mean field' produced by the binding state of the other sites. It is not clear how well this condition can be met by typical allosteric enzymes, where the number of substrate-binding sites rarely exceeds four and distances of separation are not large. It is also not clear how the 'mean field' brings about its physical results. In effect, the original assumptions proposed as the basis for the model by Ainsworth (1977) are little different. It was suggested that the measure of protein conformation most relevant to the experiment $v = f(A)$ is the constant α_i and that its value is determined by a stabilization of the protein structure directly proportional to fractional saturation. Again, no mechanism was suggested that might predict the required result.

The purpose of the present paper is to propose a physical mechanism that derives directly from eqns. (1) and (2). The general assumptions made by Ainsworth (1977) are repeated and it is taken as fact that eqns. (1) and (2) accurately represent the data to which they were applied.

THEORY

Arrangement of free-energy levels

The exponential model equation for a single ligand (eqn. 2) can be written as:

$$p_i = \frac{\ln\alpha_i - \ln\alpha_0}{\ln\alpha_1 - \ln\alpha_0} = \frac{\Delta G_0 - \Delta G_i}{\Delta G_0 - \Delta G_1} \quad (3)$$

Hence:

$$p_i = \frac{(G_0^P - G_0^R) - (G_i^P - G_i^R)}{(G_0^P - G_0^R) - (G_1^P - G_1^R)} \quad (4)$$

where the superscripts P and R respectively define the free energies in RT units of the enzyme–ligand complex and the free reagents, all at unit concentration. Eqn. (4) can be re-arranged to give:

$$p_i = \frac{(G_0^P - G_i^P) - (G_0^R - G_i^R)}{(G_0^P - G_1^P) - (G_0^R - G_1^R)} = \frac{\Delta G_i^R - \Delta G_i^P}{\Delta G_1^R - \Delta G_1^P} \quad (5)$$

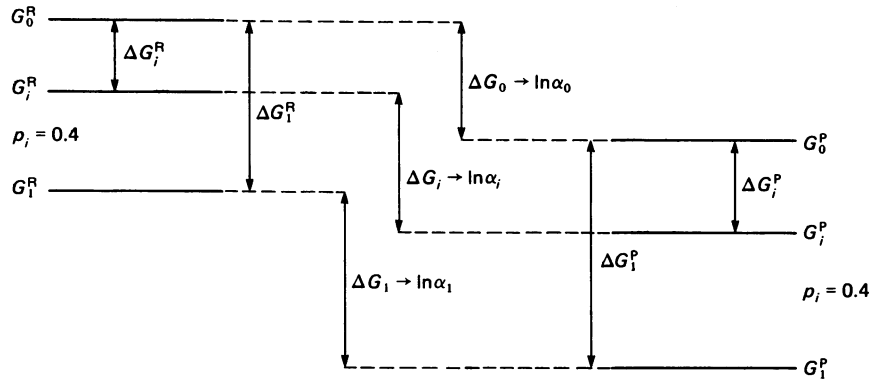

Fig. 1. Standard free energies in units of *RT* derived from eqn. (2) for the single-substrate exponential model with $a_i > 1$ and $k > 0$

where the differences are formed relative to the G_0 levels. With the definitions

$$n_i = \Delta G_i^R/\Delta G_1^R \quad \text{and} \quad m_i = \Delta G_i^P/\Delta G_1^P \tag{6}$$

eqn. (5) becomes:

$$p_i = n_i\left(\frac{\Delta G_1^R}{\Delta G_1^R - \Delta G_1^P}\right) + m_i\left(\frac{-\Delta G_1^P}{\Delta G_1^R - \Delta G_1^P}\right) \tag{7}$$

The terms within parentheses are constant with respect to p_i, and sum to 1. Hence eqn. (7) can only be true for all p_i when:

$$n_i = m_i = p_i \tag{8}$$

Further, by combining eqns. (3) and (5):

$$\ln\alpha_i - \ln\alpha_0 = p_i(\Delta G_1^R - \Delta G_1^P) \tag{9}$$

it can be seen that $\ln\alpha_i$ is different from $\ln\alpha_0$ when ΔG_1^R and ΔG_1^P are unequal.

The requirements of eqns. (8) and (9) are exemplified in Fig. 1 for the condition that $\alpha_i > 1$, $k = \ln\alpha_1 - \ln\alpha_0 > 0$.

Origin of the stable state $\ln a_i$

The basic assumption of the exponential model is that only one conformational state of the protein, represented by $\ln\alpha_i$, is stable at the fractional saturation, p_i. We now consider a possible cause for this condition.

The exponential model equation for a single ligand can be re-arranged to give:

$$p_i \cdot \ln\left(\frac{\alpha_i}{\alpha_1}\right) = (1-p_i)\cdot\ln\left(\frac{\alpha_0}{\alpha_i}\right) \tag{10}$$

But:

$$\ln\left(\frac{\alpha_i}{\alpha_1}\right) = (G_1^P - G_1^R) - (G_i^P - G_i^R)$$
$$= (G_1^P - G_i^P) - (G_1^P - G_i^R) \tag{11}$$

Hence by eqn. (8):

$$\ln\left(\frac{\alpha_i}{\alpha_1}\right) = (1-p_i)[(G_1^P - G_0^P) - (G_1^R - G_0^R)]$$
$$= (1-p_i)(\Delta G_1 - \Delta G_0) \tag{12}$$

or:

$$\ln\left(\frac{\alpha_i}{\alpha_1}\right) = (1-p_i)\cdot\ln\left(\frac{\alpha_0}{\alpha_1}\right) \tag{13}$$

By a similar transformation of $\ln(\alpha_0/\alpha_i)$, or more directly from eqn. (3), we can modify eqn. (10) to read:

$$(p_i)_t\left[(1-p_i)_c \cdot \ln\left(\frac{\alpha_0}{\alpha_1}\right)\right] = (1-p_i)_t\left[(p_i)_c \cdot \ln\left(\frac{\alpha_0}{\alpha_1}\right)\right] \tag{14}$$

where the subscript c defines the connection between fractional saturation and the conformational energy differences established by eqns. (11)–(13). The second subscript t is introduced to show that the lifetimes of the ligand-bound and ligand-free forms of the protein, in its conformation $\ln\alpha_i$, must be in proportion to the ratio $(p_i)_t/(1-p_i)_t$. Now, the stable conformations corresponding to the bound and free states of the protein are defined by $\ln\alpha_1$ and $\ln\alpha_0$ respectively; it can be concluded, therefore, that the conformation $\ln\alpha_i$ relaxes in the directions $\ln\alpha_1$ and $\ln\alpha_0$ during the time intervals $(p_i)_t$ and $(1-p_i)_t$. The form of eqn. (14) then suggests the following further conclusions. First, the ratio $(1-p_i)_c/(p_i)_c$ represents the relative rates of first-order decays of conformational energy that are equally proportional to the conformational energy differences established respectively between $\ln\alpha_i$ and the limiting conformations $\ln\alpha_1$ and $\ln\alpha_0$. Secondly, the equality $(p_i)_t \cdot (1-p_i)_c = (1-p_i)_t \cdot (p_i)_c$ requires that the lifetimes $(p_i)_t$ and $(1-p_i)_t$ must be very short in comparison with the lifetime of conformational change in order that the terms $(1-p_i)_c$ and $(p_i)_c$ may become the tangents to the curves that represent conformational relaxation at $\ln\alpha_i$ and p_i.

The interpretation of eqn. (14) as being the representation of a system in slow conformational equilibrium precludes the existence of stable protein states other than $\ln\alpha_i$ at a given p_i and therefore of the equilibria between definable co-existing states that form the basis for the Adair equation (Weber & Anderson, 1965; Weber, 1965).

The interpretation also implies that maintenance of the state $\ln\alpha_i$ at p_i is an active process. How this comes about may be examined by supposing that a second conformation $\ln\alpha_j$ exists at the ligand concentration A_t, the latter being determined by the defined values of $\ln\alpha_i$ and p_i. Eqn. (14) now becomes:

$$(p_{ji})_t\left[(1-p_j)_c \cdot \ln\left(\frac{\alpha_0}{\alpha_1}\right)\right] \neq (1-p_{ji})_t\left[(p_j)_c \cdot \ln\left(\frac{\alpha_0}{\alpha_1}\right)\right] \tag{15}$$

where p_{ji} is the fractional saturation of molecules in state

$\ln\alpha_j$ at ligand concentration A_i, defined by:

$$p_{ji} = \frac{A_i\alpha_j}{1+A_i\alpha_j} \quad \text{and} \quad A_i = \frac{p_i}{1-p_i}\cdot\frac{1}{\alpha_i} \tag{16}$$

p_j, by contrast, is the fractional saturation consistent with the conformational state $\ln\alpha_j$, and its relation to $\ln\alpha_0$ and $\ln\alpha_1$. p_j is therefore defined by analogy with eqn. (3).

Both sides of eqn. (15) represent changes in free energy. Hence when the left-hand side of the inequality is negative the protein structure relaxes towards $\ln\alpha_1$; similarly, a negative value for the right-hand side represents relaxation towards $\ln\alpha_0$ and vice versa with change of sign. With these requirements, eqn. (15) gives:

$$kp_{ji}+\ln\left(\frac{\alpha_0}{\alpha_j}\right) = R_j \underset{\ln\alpha_1}{\overset{\ln\alpha_0}{\lesseqgtr}} 0 \tag{17}$$

where R_j is a free-energy difference that takes a negative value when the net relaxation of protein structure is in the direction $\ln\alpha_0$. Hence, by comparison with the other quantities in the equation, we can write:

$$R_j = \ln\left(\frac{\alpha_k}{\alpha_j}\right) \tag{18}$$

where $\ln\alpha_k$ is the state produced from $\ln\alpha_j$ because of the incompatability of $\ln\alpha_j$ and A_i. It is easily shown by calculation that for:

$$\ln\left(\frac{\alpha_j}{\alpha_i}\right) \gtrless 0,\ R_j \lesseqgtr 0,\ \ln\left(\frac{\alpha_k}{\alpha_j}\right) \lesseqgtr 0 \tag{19}$$

Eqn. (19) shows that protein conformations with $\ln\alpha_j \gtrless \ln\alpha_i$ undergo a structural change corresponding to a movement to state $\ln\alpha_k$ where $\ln\alpha_j \gtrless \ln\alpha_k \gtrless \ln\alpha_i$. As a result, in the absence of countervailing forces, all molecules originally in the state $\ln\alpha_j$ return to state $\ln\alpha_i$ by a series of consecutive transformations $\ln\alpha_j \rightarrow \ln\alpha_k \rightarrow \ln\alpha_l \dashrightarrow \ln\alpha_i$. Eqn. (19) therefore establishes $\ln\alpha_i$ as the only stable state of the protein when randomization forces are assumed absent. The stabilization energy for the transformation $\ln\alpha_j$ into $\ln\alpha_k$ then becomes:

$$S_j = -|R_j| \tag{20}$$

Distribution of protein molecules in states $\ln\alpha_j$

In the steady state of the system S_j must be equal and opposite to the energy of randomization, T_j, which creates state $\ln\alpha_j$ from $\ln\alpha_k$. [Randomization must inevitably occur because the quantities $(p_i)_t$ and $(1-p_i)_t$ for individual molecules have values distributed about the averages for the assembly.] If it is assumed that T_j and S_j take place consecutively and independently it can be concluded that the n_j molecules established by T_j have a conformation $\ln\alpha_k$ established by S_j. Correspondingly, n_k molecules have a binding constant of α_l and so on. The relative number of molecules in the $\ln\alpha_j$ and $\ln\alpha_k$ states is calculated by assuming a Boltzmann distribution:

$$\frac{n_j}{n_k} = \exp(-T_j) \tag{21}$$

However, to calculate the overall distribution of n_j it is more convenient to normalize with respect to $n_i = 1$, i.e.:

$$n_j = \exp\left[-\left|\ln\left(\frac{\alpha_j}{\alpha_k}\cdot\frac{\alpha_k}{\alpha_i}\right)\right|\right] \tag{22}$$

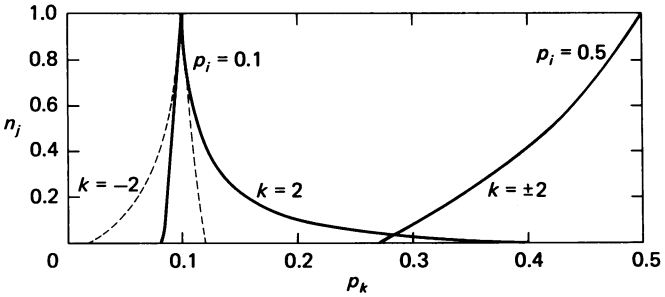

Fig. 2. Number of molecules n_j, with fractional saturation p_k obtained when the corresponding monodisperse system is described by A_i, p_i and $\ln\alpha_i$, with $\ln\alpha_0 = 0$ and $k = +2$ or -2 (eqn. 2)

The distribution of n_j for values of p_k greater than 0.5 is obtained by reflecting the Figure about the vertical axis through $p_k = 0.5$.

Table 1. Values of the exponential model constants $\ln\alpha_0$ and k calculated from $\ln\bar{\alpha} = f(\bar{p})$ where the data were produced by the distribution discussed in the text, obtained with $p_i = 0.1$ to 0.9, step 0.1, and the true constants given in the Table

The measured constants given in the Table also provide the lines:

$k_{(true)} = 1.158\ (\pm 0.008)\cdot k_{(measured)} + 0.028\ (\pm 0.014)$

$\ln\alpha_{0(measured)} = 0.079\ (\pm 0.004)\cdot k_{(measured)} + 0.014\ (\pm 0.007)$

True values		Measured values ± S.D.	
$\ln\alpha_0$	k	$\ln\alpha_0$	k
0	3.9	0.247±0.027	3.406±0.050
	3.0	0.232±0.008	2.536±0.014
	2.0	0.171±0.005	1.657±0.009
	1.5	0.130±0.006	1.239±0.011
	1.0	0.086±0.005	0.827±0.010
	0.5	0.042±0.003	0.416±0.006
	−0.5	−0.038±0.003	−0.424±0.006
	−1.0	−0.070±0.006	−0.859±0.010
	−1.5	−0.096±0.007	−1.308±0.013
	−2.0	−0.114±0.007	−1.771±0.012

Fig. 2 illustrates distributions $n_j = f(p_k)$ calculated by a simple program based on eqns. (16)–(22). The calculation depends on the assumption that the protein has access to an indefinite span of conformational energies, linearly related to $\ln\alpha_j$, but demonstrates that the S_j transformation brings all molecules into the required conformational range $\ln\alpha_0$ to $\ln\alpha_1$ when the steady state is achieved.

Experimental determination of the parameters of the single-ligand exponential model

The determination of $\ln\alpha_0$ and k for the single-substrate exponential model depends on a plot of the relationship between $\ln\bar{\alpha}_i$ and $\bar{p}_i$, where $\bar{p}_i$ is the observed fractional saturation at ligand concentration A_i and:

$$\ln\bar{\alpha}_i = \ln\left(\frac{\bar{p}_i}{1-\bar{p}_i}\cdot\frac{1}{A_i}\right) \tag{23}$$

The program described above calculates values of the averages $\ln \bar{\alpha}$ and $\bar{p}$, corresponding to defined values of p_i, k and $\ln \alpha_0$, on the assumption that the number of molecules in state $\ln \alpha_k$ is given by n_j as described in the preceding section. Table 1 then gives the line constants of the relationships $\ln \bar{\alpha} = f(\bar{p})$, for $p_i = 0.1$ to 0.9, step 0.1, at different values of k with $\ln \alpha_0$ set to zero. It will be observed throughout that $\ln \bar{\alpha}$ is a linear function of $\bar{p}$ but that the calculated values of k are some 16% smaller than the true values. Again, as k increases, the calculated values of $\ln \alpha_0$ progressively diverge from zero. (The Table legend gives the linear regression of $k_{(true)}$ on $k_{(measured)}$ and of $\ln \alpha_{0(measured)}$ on $k_{(measured)}$. Good linear relationships are observed.)

The calculations undertaken in this section show clearly that a distribution of protein conformations consistent with the model does not hide the basic relationship that was predicated on a monodisperse system.

DISCUSSION

The interpretation of the exponential model equations, developed in the preceding section, depends on several assumptions. These are now discussed in turn.

The protein has distinct structures in the ligand-bound and ligand-free states

This assumption requires little justification, supported as it is by the detailed structural analysis of oxy- and deoxy-haemoglobin (Perutz, 1970) and by the generality of its application (Monod *et al.*, 1965; Koshland *et al.*, 1966).

The protein undergoes continuous conformational change

The essential assumption embodied in eqn. (14) is that the conformation of the protein can undergo continuous change in passing between the limits corresponding to $\ln \alpha_0$ and $\ln \alpha_1$. Ample evidence exists that proteins have the flexible structures necessary to achieve the change (Englander *et al.*, 1972; Artymiuk *et al.*, 1979; Frauenfelder *et al.*, 1979; McCammon *et al.*, 1979; Beece *et al.*, 1980; Karplus & McCammon, 1986), and flexibility is central to commonly held views of enzyme action (Koshland, 1958). Furthermore, the equilibria between protein tautomeric species, postulated as the basis for the Adair binding equation (Weber & Anderson, 1965), must shade into a structural relaxation continuous with p_i as the number of tautomers increases. Differentiation between the two schemes is finally a matter of semantics (Huber, 1979).

Long conformational lifetimes

The critical assumption that therefore differentiates eqns. (1) and (2) from an Adair model is the statement that the enzyme takes much longer to relax from one limiting structure to the other than it does to change its binding state (Weber, 1965; Weber & Anderson, 1965).

Support for the assumption can be mustered from two directions, that is by considering factors that either lengthen the lifetime of structural relaxation or shorten the lifetimes of the free and bound states. In both cases the oligomeric structure of the protein appears to be important.

There is nothing in the development of the exponential model to prevent its application to monomeric proteins, yet it is clear that regulatory function is almost completely limited to oligomeric enzymes. To this point, Weber (1975) has related the rate of appearance of a protein conformation to the number of non-covalent bonds of average energy that have to be broken in the process. Thus it can be shown that structural fluctuations involving eight to ten amino acid residues take place only once a second. This condition is more likely to arise when structural effects originating in protein subunits are applied across common boundaries. In Weber's (1975) view, the resulting increase in the energy difference between the liganded and ligand-free states, with its opportunities for modulation, is the cause of the evolution of multi-chain proteins.

Again, with regard to the shortening of the lifetimes of the ligand-bound and ligand-free states of the protein, it is likely that structure is important. It has been suggested that, because of van der Waals adsorption, the concentration of substrate molecules on the surface of an enzyme is much larger than that in the bulk solution (Zhou & Zhong, 1982). If so, estimates of the rates of dissociation, dependent on the estimation of the bulk concentration of ligands, may well be too low. Correspondingly, rates of association will be greater than expected. These effects are likely to be more important with oligomeric enzymes than with simple monomers because of the increase in the number of binding sites disposed on or near the adsorbing surface of the protein. Indeed, it is considerations of this sort that underpin the assumption of benefit that is supposed to result from the aggregation of several enzymes into structurally defined complexes or loose associations (Welch, 1977; Gaertner, 1978; Keleti, 1984).

Quasi-equilibrium

The interpretation of the exponential model as essentially a steady-state system indicates that the determination of affinity constants by eqn. (23) and their relation to free-energy changes can only be justified by the assumption of quasi-equilibrium, that is, by the assumption that the binding and dissociation reactions of the enzyme–ligand system are fast in comparison with the rate at which structural relaxation of the enzyme changes its ligand affinity. A quasi-equilibrium assumption, however, has already been invoked in order to relate the initial velocities of the enzyme-catalysed reaction to the fractional saturation of the enzyme by its substrates (Ainsworth, 1977). Its extended application therefore requires the rate of product formation to be lower than the rate at which the enzyme changes its structure with saturation. In this connection, it is interesting to note that protein relaxation may also be involved in the enzyme catalysis of substrate reaction, as distinct from substrate binding (Blumenfeld, 1976): if so, the assumption that has been made requires the first type of relaxation to be slower than the second.

Conclusion

The purpose of this paper has been to establish that the exponential model offers a plausible explanation of regulatory behaviour, the more important because it is, in comparison with other alternatives, parsimonious in its employment of disposable constants (Ainsworth, 1977; Morris *et al.*, 1986). For all that, no proof of its validity has been provided. The combination of a suitable equation and, when several ligands are involved, a more

than adequate number of constants is usually sufficient to ensure that a reasonable fit to data is achieved. It is therefore likely that a more searching examination of validity will turn on the determination of the lifetimes involved: for example, support would be provided if the rate of conformational change were shown to be distinctly less than the rates of binding and release of the ligand by the protein.

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